IN THE CLAIMS:

1. - 87. (Canceled)

88. (Currently Amended) A method of assaying an enzyme in a sample of blood or blood derivative, comprising the steps of:

introducing the sample into a cartridge which includes an analysis location,

metering a portion of the sample,

moving the metered sample to the analysis location,

mixing the metered sample with reagent at the analysis location,

allowing the enzyme to react with the reagent to form a reaction product in the sample,

positioning the ~~reacted~~ sample at a sensor in the analysis location, and

detecting the reaction product of the enzyme reaction using the sensor.

89. (Canceled)

90. (New) A method of detecting a reaction product in a sample of blood or blood derivative using a cartridge that includes a holding chamber, an overflow chamber, an analysis location, a pump, a reagent and a sensor, the method comprising the steps of:

(a) introducing the sample into the holding chamber in the cartridge;

(b) metering a portion of the sample by retaining excess sample in the overflow chamber;

(c) moving the metered sample from the holding chamber to the analysis location by means of the pump;

(d) mixing the metered sampled with the reagent in the analysis location;

(e) allowing the reagent to form the reaction product in the sample;

(f) positioning the sample at the sensor in the analysis location using the pump; and

(g) detecting the reaction product at the sensor.

91. (New) The method of claim 90, wherein the excess sample passes through a substantially circular orifice located between the holding chamber and the overflow chamber.

92. (New) The method of claim 90, wherein a capillary stop is located between the holding chamber and the analysis location.

93. (New) The method of claim 90, wherein a capillary stop is located between the holding chamber and the analysis location,

wherein the excess sample passes through a substantially circular orifice located between the holding chamber and the overflow chamber, and

wherein the area of the substantially circular orifice is larger than the area of the capillary stop.

94. (New) The method of claim 90, wherein a capillary stop is located between the holding chamber and the analysis location,

wherein the excess sample passes through a substantially circular orifice located between the holding chamber and the overflow chamber, and

wherein the substantially circular orifice comprises a lower resistance to fluid sample flow than the capillary stop.

95. (New) The method of claim 90, wherein a capillary stop is located between the holding chamber and the analysis location,

wherein a substantially circular orifice is located between the holding chamber and the overflow chamber, and

wherein the volume of the holding chamber between the substantially circular orifice and the capillary stop corresponds substantially to the metered volume of the fluid sample.

96. (New) The method of claim 90, wherein the overflow chamber receives excess sample from the holding chamber through an orifice.

97. (New) The method of claim 90, comprising the step of:

(h) sealing the holding chamber with a closable sample entry port after step (a).

98. (New) The method of claim 97, wherein the excess sample is forced into the overflow chamber by closure of the closable sample entry port.

99. (New) The method of claim 97, wherein a circumferential well around the closable sample entry port receives spilled sample.

100. (New) The method of claim 97, wherein the closable sample entry port comprises an air-tight seal when closed.

101. (New) The method of claim 90, wherein the cartridge includes a capillary stop and a pre-sensor chamber located between the holding chamber and the analysis location.

102. (New) The method of claim 90, wherein step (c) causes the sample to pass through a hydrophobic region.

103. (New) The method of claim 90, wherein the cartridge includes a hydrophobic area between the holding chamber and analysis location.

104. (New) The method of claim 103, wherein the hydrophobic area comprises a hydrophobic matrix coating selected from the group consisting of wax, petroleum gel, and non-polar organic film.

105. (New) The method of claim 103, wherein the hydrophobic area comprises a layer of material selected from the group consisting of polytetrafluoroethylene, plastic coated with polytetrafluoroethylene, polyimide treated with a fluoride ion-plasma, silicon dioxide coated with an organic compound, an alloy of tungsten and titanium, and silver coated with silver chloride.

106. (New) The method of claim 103, wherein the hydrophobic area comprises a layer of polytetrafluoroethylene.

107. (New) The method of claim 90, wherein the overflow chamber includes walls that are wetted when excess sample enters the overflow chamber.

108. (New) The method of claim 90, wherein wall surfaces of the holding chamber are corona treated.

109. (New) The method of claim 90, wherein the volume of the sample is in the range of about 1 microliter to about 1 milliliter.

110. (New) The method of claim 90, wherein the volume of the sample is in the range of about 20 microliters to about 50 microliters.

111. (New) The method of claim 90, wherein the volume of the overflow chamber is in the range of about 0.2 microliters to about 1 milliliter.

112. (New) The method of claim 90, wherein the volume of the overflow chamber is in the range of about 1 microliter to about 10 microliters.

113. (New) The method of claim 90, wherein the pump is in fluidic connection with

the overflow chamber.

114. (New) The method of claim 90, wherein a force provided to the sample by the pump comprises a pneumatic force.

115. (New) The method of claim 90, wherein at least a portion of at least one of the holding chamber and the overflow chamber is treated to impart a high energy surface to interior chamber surfaces.

116. (New) The method of claim 90, wherein the cartridge is adapted for use with an analyzer.

117. (New) The method of claim 116, wherein the pump is actuated by an actuator element of the analyzer.

118. (New) The method of claim 90, wherein the reagent comprises a predetermined amount of reagent in the analysis location for mixing with the sample.

119. (New) The method of claim 90, wherein interior surfaces of at least one of the holding chamber and the overflow chamber are corona treated.

120. (New) The method of claim 90, wherein the holding chamber comprises a lower interior-surface-to-volume ratio than the overflow chamber.

121. (New) The method of claim 90, wherein the reagent comprises a predetermined amount of reagent in the holding chamber for mixing with the sample.

122. (New) The method of claim 90, wherein the analysis location comprises one or

more sensors.

123. (New) The method of claim 90, wherein the reaction product is detected by an optical sensor.

124. (New) The method of claim 90, wherein the reaction product comprises an electrochemical species detected by an electrochemical sensor.

125. (New) The method of claim 90, wherein the reaction product is formed by an enzyme in the sample, and

wherein the reaction product is selected from the group consisting of factor VII, factor VIII, factor IX, factor X, factor XI, factor XII, and thrombin.

126. (New) The method of claim 90, wherein the reaction product is formed by the enzyme thrombin.

127. (New) The method of claim 90, wherein the reagent includes solubility-enhancing components.

128. (New) The method of claim 90, wherein the reagent includes an electrochemical species other than a substrate and its reaction product.

129. (New) The method of claim 128, wherein the electrochemical species is detectable at a different electrical potential than the substrate of the product.

130. (New) The method of claim 90, wherein the reagent comprises an enzyme substrate deposited at more than one site within the analysis location.

131. (New) The method of claim 90, wherein the sample is oscillated by the pump over a first and a second sensor while in the analysis location.

132. (New) The method of claim 90, wherein the sensor measures the concentration of reaction product each time the fluid sample is oscillated passed the sensor by the pump.

133. (New) The method of claim 90, wherein the reagent comprises a matrix that promotes rapid dissolution into the sample.

134. (New) The method of claim 90, wherein the reagent comprises at least one component selected from the group consisting of a water-soluble polymer, gelatin, agarose, a polysaccharide, polyethylene glycol, polyglycine, a saccharide, sucrose, an amino acid, glycine, a buffer salt, sodium phosphate, HEPES buffer, and a dye molecule.

135. (New) The method of claim 90, wherein the reagent promotes the coagulation of blood or blood derivative.

136. (New) The method of claim 90, wherein the reagent is selected from the group consisting of celite, kaolin, diatomaceous earth, clay, silicon dioxide, ellagic acid, natural thromboplastin, recombinant thromboplastin, phospholipid, and mixtures thereof.

137. (New) The method of claim 90, wherein the sensor comprises a first sensor comprising a conductimetric sensor, and a second sensor comprising an amperometric sensor.

138. (New) The method of claim 137, wherein the amperometric sensor comprises an applied potential of about +0.4V versus a silver-silver chloride electrode.

139. (New) The method of claim 137, wherein the amperometric sensor comprises an

applied potential of about +0.1V versus a silver-silver chloride electrode.

140. (New) The method of claim 137, wherein the conductimetric sensor is proximal to the holding chamber, and

wherein the amperometric sensor is distal from the holding chamber.

141. (New) The method of claim 137, wherein one of the first and second sensors is comprised of a metal selected from the group consisting of gold, platinum, silver, and iridium.

142. (New) The method of claim 137, wherein one of the first and second sensors is coated with a self-assembled thiol film.

143. (New) The method of claim 137, wherein one of the first and second sensors is in the shape of an antenna.

144. (New) The method of claim 90, wherein the cartridge operates in conjunction with an analyzer,

wherein the analyzer applies a potential to the sensor with the generation of an electrochemical signal, and

wherein the signal is proportional to the concentration of a substrate reagent in the sample.

145. (New) The method of claim 90, wherein the cartridge operates in conjunction with an analyzer,

wherein the analyzer applies a potential to the sensor with the generation of an electrochemical signal, and

wherein the signal is proportional to the concentration of a product of a reagent substrate in the sample.

146. (New) The method cartridge of claim 90, wherein the reaction product in the sample is produced by the enzyme thrombin and the reagent comprises a thrombin substrate, and

wherein hydrolysis of the substrate by thrombin forms a product that reacts at the sensor with the generation of a signal distinguishable from a signal generated by the substrate.

147. (New) The method of claim 90, wherein the sensor comprises a microfabricated amperometric sensor.

148. (New) The method of claim 90, wherein the sensor comprises a microfabricated conductimetric sensor.

149. (New) The method of claim 90, wherein the sensor comprises a first and a second sensor,

wherein the first sensor comprises a microfabricated conductimetric sensor, and
wherein the second sensor comprises a microfabricated amperometric sensor.

150. (New) The method of claim 90, wherein the reagent includes a substance that promotes coagulation of the sample.

151. (New) The method of claim 90, wherein the sample is selected from the group consisting of blood containing one of an additive and a diluent, plasma, serum, plasma containing one of an additive and a diluent, and serum containing one of an additive and a diluent.

152. (New) The method of claim 90, wherein the reagent comprises a substrate that is selected from the group consisting of a tosyl-glycyl-prolinyl-arginyl- moiety, H-D-phenylalanyl-pipecolyl- moiety, and benzyl phenylalanyl-valyl-arginyl- moiety attached to a moiety selected

from the group consisting of an N-phenyl-p-phenylenediamine moiety, and an N-[p-methoxyphenyl-]-p-phenylenediamine moiety.

153. (New) The method of claim 90, wherein the reaction product is selected from the group consisting of N-phenyl-p-phenylenediamine moeity and N-[p-methoxyphenyl-]-p-phenylenediamine moiety.

154. (New) The method of claim 90, wherein the reaction product is produced by an enzyme in the sample.

155. (New) The method of claim 90, wherein the reaction product is produced by an enzyme selected from the group consisting of glucose oxidase, lactate oxidase, and other oxidoreductases, dehydrogenase based enzymes, alkaline phosphatase and other phosphatases, and serine proteases.

156. (New) The method of claim 90, wherein the sensor is coated with a mercaptoalkanol reagent selected from the group consisting of mercaptoethanol, mercaptopropanol, mercaptobutanol, and mixtures thereof.

157. (New) The method of claim 90, wherein the reagent is deposited in the path of the moving sample of step (c).

158. (New) The method of claim 90, wherein the pump oscillates the sample in the analysis chamber with the trailing edge of the sample positioned in the region of a selected sensor to dissolve the substrate in that portion of the sample near the trailing edge.

159. (New) The method of claim 158, wherein the oscillation comprises a frequency in the range of about 0.2 Hz to about 10 Hz for a period in the range of about 1 second to about

100 seconds.

160. (New) The method of claims 158, wherein the oscillation comprises a frequency in the range of about 1.5 Hz for a period of about 20 seconds.

161. (New) The method of claim 158, wherein the oscillation comprises a frequency of about 0.3 Hertz, and

wherein the sensor generates a signal at each oscillation.

162. (New) The method of claim 158, wherein the oscillation comprises a frequency sufficient to prevent settling of erythrocytes on the sensor.

163. (New) The method of claim 158, wherein the sensor comprises an amperometric sensor, and

wherein the method further comprises the step of:

(h) storing a first sensor signal after a reagent is dissolved.

164. (New) The method of claim 163, further comprising the step of:

(i) analyzing subsequent amperometric sensor signals to determine a maximum rate of change in sensor signal.

165. (New) The method of claim 163, further comprising the step of:

(i) determining a fixed fraction of a maximum rate of change in the sensor signal.

166. (New) The method of claim 165, further comprising the step of:

(j) determining a coagulation parameter from the first sensor signal and the fixed fraction.

167. (New) A method of assaying an enzyme in a sample of blood or blood derivative using a cartridge that includes a holding chamber, an overflow chamber, an analysis location, a pump, a reagent and a sensor, the method comprising the steps of:

(a) introducing the sample into the holding chamber in the cartridge;

(b) metering a portion of the sample by retaining excess sample in the overflow chamber;

(c) moving the metered sample from the holding chamber to the analysis location using the pump;

(d) mixing the metered sampled with the reagent at the analysis location;

(e) allowing the enzyme to react with the reagent to form a reaction product in the sample;

(f) positioning the sample at the sensor in the analysis location using the pump; and

(g) detecting the reaction product of the enzyme reaction at the sensor.

168. (New) A method of determining a coagulation parameter in a sample using a single-use cartridge in combination with an analyzer, wherein the cartridge includes an entry port for receiving a sample, an entry port closure, a holding chamber in communication at a first end with the entry port, and a capillary stop in communication with the holding chamber at a second end, wherein the capillary stop is in communication with an analysis chamber containing a reagent, a conductimetric sensor and an amperometric sensor, wherein the holding chamber is in communication with an overflow chamber for receiving and retaining excess sample, wherein the overflow chamber is in communication with a pneumatic pump actuated by the analyzer, wherein the analyzer is capable of actuating the pneumatic pump to displace the sample from the holding chamber, the method comprising the steps of:

(a) applying the sample to the entry port;

(b) filling the holding chamber;

(c) closing the entry port;

(d) permitting excess sample to enter and be retained in the overflow chamber;

(e) inserting the cartridge into the analyzer;

(f) actuating the pneumatic pump to deliver a metered portion of the sample into the analysis chamber;

(g) reacting the sample with the reagent in the analysis chamber;

(h) positioning the sample in the analysis chamber with respect to the conductimetric sensor;

(i) detecting a reaction product from the reagent at the amperometric sensor; and

(j) determining the coagulation parameter from an output of the amperometric sensor.

169. (New) The method of claim 168, wherein the coagulation parameter is selected from the group consisting of prothrombin time, activated partial thromboplastin time, activated clotting time, kaolin activated clotting time and celite activated clotting time.

170. (New) The method of claim 168, wherein in step (h) the sample is oscillated back and forth by the pneumatic pump using the output of the conductimetric sensor.

171. (New) The method of claim 168, wherein the cartridge comprises a hydrophobic area located between the analysis location and the holding chamber.

172. (New) The method of claim 168, wherein the volume of the sample is in the range of about 1 microliter to about 1 milliliter.